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㉒ N-terminal fragments of human serum albumin.

㉓ Polypeptides corresponding to mature human serum albumin residues 1 to n, where n is between 369 and 419 inclusive, are useful as substitutes for albumin in the treatment of burns and shock in humans, the clearances of undesirable compounds, (such as bilirubin) from human blood, in laboratory growth media and in HSA assays.

㉔ HSA (1-389) is particularly preferred, although not novel *per se*.
The polypeptides may be produced by recombinant DNA techniques, especially in yeast.

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POLYPEPTIDES

This invention relates to a novel polypeptide molecule which can be produced by recombinant DNA technology and can be used for many of the existing applications of human serum albumin.

Human serum albumin (HSA) is the most abundant plasma protein, contributing 60% w/w of the total protein content of the plasma. A molecule of HSA consists of a single non-glycosylated polypeptide chain of 585 amino acids of formula molecular weight 66,500. The amino acid sequence of HSA has been established by protein sequence analysis (Meloun et al, 1975, "Complete amino acid sequence of human serum albumin" FEBS Letters: 58:1, 136-317; Behrens et al, 1975, "Structure of human serum albumin" Fed. Proc. 34, 591) and more recently by genetic analysis (Lawn et al, 1981, Nucleic Acids Research 9, 6102-6114). Although there have been discrepancies between the amino acid sequences as published (some being attributable to polymorphisms), Figure 1 represents the amino acid sequence currently believed to be most representative of the HSA present within the human population.

Because of its relatively small molecular weight and net negative charge at physiological pH (Peters, 1970, "Serum albumin", Adv. Clin. Chem. 13, 37-111), HSA contributes 85% of the osmotic effect of normal plasma. Thus HSA is the principal regulator of plasma volume. A secondary role of HSA is to bind small molecules produced by catabolic processes (for example fatty acids and bilirubin). Albumin represents the principal means for the transport of these key metabolites, which are poorly soluble at physiological pH. Physical, chemical, immunological and limited proteolytic studies of HSA have shown that the molecule is composed of regions of polypeptide chains which retain their conformation after separation from the parent molecule by enzymatic means. These polypeptide chains retain their binding capabilities thereby facilitating the mapping of binding sites for bilirubin, fatty acids and other small molecules to particular regions of the polypeptide chain (Kragh-Hansen, 1981, "Molecular aspects of ligand binding to serum albumin". A. Soc. Pharm. Expt. Ther. 33, 1, 17-53). Much of the information in this area has been reviewed (Brown and Shockley, 1982, "Serum albumin: structure and characterisation of its ligand binding sites").

The indications for the clinical use of therapeutic concentrates of HSA are related principally to its 25 oncotic action as a plasma volume expander. Concentrates of HSA have been used therapeutically since the 1940's, in particular in cases of shock, burns, adult respiratory distress syndrome, and cardiopulmonary bypass. Albumin has also been used in cases of acute liver failure, following removal of ascitic fluid from patients with cirrhosis, after surgery, in acute nephrosis, in renal dialysis, and as a transport protein for removing toxic substances, such as in severe jaundice in haemolytic disease of the new born.

30 In addition to its use as a therapeutic agent, HSA is a major component of serum added to media used to support the growth of mammalian cells in tissue culture. The consumption of serum and hence of albumin has been greatly increased over recent years as biotechnology and pharmaceutical companies have expanded their tissue culture for research and for production. There is a universal need for lower cost and better regulation of sera for these purposes.

35 It is known to manipulate the HSA-encoding DNA sequence express a recombinant polypeptide in microorganisms. Indeed such a recombinant HSA polypeptide has been produced in bacterial species such as Escherichia coli (G.B. Patent No. 2 147 903B) and Bacillus subtilis (European Patent Application No. 86304656.1) and the yeast Saccharomyces cerevisiae (European Patent Publication No. 201 239, Delta Biotechnology Ltd.); thus it is generally accepted that a recombinant polypeptide essentially identical to 40 natural HSA can be produced in a variety of microbial hosts by employing known methods. However, in all cases where recombinant HSA has been produced, the objective has been to produce a molecule which is "nature-identical" to HSA in structure and biological function.

It has now been found that it is advantageous to produce shorter forms of HSA.

45 One aspect of the present invention provides a polypeptide comprising the N-terminal portion of human serum albumin up to amino acid residue n, where n is 369 to 419, and variants thereof.

The novel polypeptides of the invention are hereinafter referred to as "HSA(1-n)".

The terms "human serum albumin" is intended to include (but not necessarily to be restricted to) known or yet-to-be discovered polymorphic forms of HSA. For example, albumin Naskapi has Lys-372 in place of Glu-372 and pro-albumin Christchurch has an altered pro-sequence. The term "variants" is 50 intended to include (but not necessarily to be restricted to) minor artificial variations in residues 1 to n (such as molecules lacking one or a few residues, having conservative substitutions or minor insertions of residues, or having minor variations of amino acid structure). Thus polypeptides which have 80%, preferably 85%, 90%, 95% or 99%, homology with any HSA (1-n) compound are deemed to be "variants". Such variants are preferably 360 to 430 amino acids long, more preferably 369 to 419 amino acids long and most preferably 386 to 388 amino acids long. It is also preferred for such variants to be physiologically equivalent

to HSA (1-n) compounds; that is to say, variants preferably share at least one pharmacological utility with HSA (1-n) compounds. Furthermore, any putative variant which is to be used pharmacologically should be non-immunogenic in the animal (especially human) being treated.

5 Conservative substitutions are those where one or more amino acids are substituted others having similar properties such that one skilled in the art of polypeptide chemistry would expect at least the secondary structure, and preferably the tertiary structure, of the polypeptide to be substantially unchanged. For example, typical such substitutions include alanine or valine for glycine, arginine or asparagine for glutamine, serine for asparagine and histidine for lysine. Variants may alternatively, or as well, lack up to ten (preferably only one or two) amino acid residues in comparison with any given HSA (1-n); preferably any 10 such omissions occur in the 100 to 369 portion of the molecule (relative to mature HSA itself). Similarly, up to ten, but preferably only one or two, amino acids may be added, again in the 100 to 369 portion for preference. The term "physiologically functional equivalents" also encompasses larger molecules comprising the said 1 to n sequence plus a further sequence at the N-terminal (for example, pro-HSA(1-n), pre-pro-HSA(1-n), met-HSA(1-n), and HSA(1-n) having a suitable leader sequence which is not necessarily native to 15 HSA).

15 If the HSA (1-n) is to be prepared by culturing a transformed yeast (*S. cerevisiae*) as is described in more detail below, the leader sequence may, for example, be that found naturally with the yeast alpha-factor protein. C-terminal fusion products with other polypeptides of interest may be produced. Known forms and fragments of HSA are clearly to be regarded as excluded from the above definition, for example 20 HSA(1-387), which was a peptic fragment produced in low yield (Geisow and Beaven, Biochem. J. 161, 619-624, 1977 and *ibid.* 163, 477-484, 1977. These prior articles identify the fragment as 1-386, but it has since become apparent (see, for example, Lawn et al, *op-cit.*) that this is due to the authors' use of incorrect published sequence information and that the fragment was in fact 1-387). Similarly, a C-terminal fusion protein comprising HSA (1-n) and the remaining HSA residues (numbers n+1 to 585) is not claimed as part 25 of the invention.

25 Particularly preferred novel HSA(1-n) compounds include HSA(1-373) (i.e. C-terminal Val), HSA(1-388) (i.e. C-terminal Ile), HSA(1-389) (i.e. C-terminal Lys), HSA(1-390) (i.e. C-terminal Gln) and HSA(1-407) (i.e. C-terminal Leu).

30 The HSA(1-n) molecules are preferably produced by means for recombinant DNA technology (optionally followed by proteolytic digestion), rather than by chemical or enzymatic degradation of natural HSA, or by peptide synthesis. In the case of enzymatic degradation, for example, a trypsin-like enzyme will cleave HSA between Lys(389) and Gln(390) but also concomitantly at other cleavage sites. In the future, peptide synthesis may become more feasible for molecules as long as 419 amino acids, but at present is not a practical proposition. Expression in yeast is particularly preferred.

35 It has been found that, at least in some situations where the HSA(1-n) compound is produced by culturing a transformed host, some HSA(1-n) compounds which are longer than HSA(1-387) are proteolytically digested back to HSA (1-387) by the enzymes which are naturally present in the system. Thus, one can, if desired, use a nucleotide sequence corresponding to a given HSA(1-n) compound in order to prepare another HSA(1-n) compound.

40 The new molecules herein described can be used as an effective substitute for either natural HSA or nature-identical recombinant HSA as a plasma volume expander. An advantage of HSA(1-n) over natural HSA and recombinant nature-identical HSA relates to the efficacy of raising the colloid osmotic pressure of blood. The smaller molecular weight (approximately 44 kilo-daltons) of the protein of the present invention means that an individual protein dose of only one-half to two-thirds that of natural-HSA or nature-identical 45 recombinant HSA will be required for the equivalent colloid osmotic effect. Consequently, any process for the production of this novel polypeptide by means of recombinant DNA technology may afford significant economic advantages over known processes for the production of nature-identical recombinant HSA, since substantially less proteinaceous material is required to be produced for an effective dose.

45 Thus, a second aspect of the invention provides a pharmaceutical composition comprising HSA(1-n)-plus, where HSA(1-n)plus is HSA(1-n) as defined above or any HSA(1-n) molecules which are known per se but have not been proposed for pharmaceutical use:

50 HSA (1-387) which, as discussed above, was a fragment produced by chance in a prior art peptic digest of HSA, is particularly preferred as the HSA(1-n) plus in such a pharmaceutical composition. The composition may comprise "variants" of HSA (1-387) as defined above.

55 A third aspect provides a method of treating a human for shock, burns or other conditions in which albumin is indicated, comprising administering intravenously a blood-bulking or blood-clearing effective non-toxic amount of a sterile non-pyrogenic solution of a polypeptide comprising HSA(1-n) plus.

Further aspects of the invention include (a) vectors, plasmids and transformed microorganisms,

including cell lines, encoding HSA(1-n)plus expression; (b) processes for the production of HSA(1-n)plus comprising the fermentation under suitable conditions of a microorganism (including a cell line) so transformed as to express HSA(1-n)plus; and (c) laboratory media comprising HSA(1-n)plus.

A further advantage of at least some HSA(1-n)plus molecules over nature-identical recombinant HSA is that their smaller size and thus reduced amino acid content has been found to lead to an increase in the yield obtained (molecules per cell dry weight) in microbial hosts relative to that obtained currently for nature-identical recombinant HSA. Thus, not only has it been found that the scale of the process can be reduced, but also productivity in the recombinant host organism can be enhanced.

The compounds of the invention may be used as blood-bulking (plasma-expanding) agents in analogous ways and in analogous formulations as HSA itself except that the dose of the HSA(1-n)plus compound (in terms of weight) will generally be less than that of HSA as the oncotic effect of the former is greater. The pharmacist or clinician skilled in the art will readily be able to determine by routine and non-inventive experimentation the optimum dose of the HSA(1-n)plus compound. Generally, the amount of HSA(1-n)plus which is administered will be about two-thirds of the amount of HSA which would be administered.

HSA (1-n) plus compounds may also be used as:

(1) substitutes for HSA or, more commonly, bovine serum albumin (BSA) in tissue culture media, thereby reducing the risk of contamination of the medium with, for example, viruses and mycoplasmas; (2) substitutes for BSA in the stationary phase in liquid chromatography for resolution of enantiomers and so on.

20

EXAMPLES

25 The invention will now be illustrated by way of example and with reference to the drawings, in which:

Figure 1 depicts the amino acid sequence currently thought to be the most representative of natural HSA, with (boxed) the alternative C-terminal of HSA(1-n);

Figure 2 depicts the DNA sequence coding for mature HSA;

Figure 3 illustrates, diagrammatically, the construction of mHOB16;

30 Figure 4 illustrates, diagrammatically, the construction of pHOB31; and

Figure 5 is a copy of a rocket electrophoretogram showing the increased yield of HSA(1-389) over complete HSA.

35 Standard recombinant DNA procedures are as described by Maniatis *et al* (1982) unless otherwise stated. Construction and analysis of M13 recombinant clones was as described by Messing (1983) and Sanger *et al.* (1977).

40 The human serum albumin coding sequence used in the construction of the following molecules is derived from the plasmid M13mp19.7 (European Patent Application No. 201 239, Delta Biotechnology Ltd.) or by synthesis of oligonucleotides equivalent to parts of this sequence. Oligonucleotides were synthesised using phosphoramidite chemistry on an Applied Biosystems 380B oligonucleotide synthesizer according to the manufacturer's recommendations (AB Inc., Warrington, Cheshire, England).

Example 1: HSA (1-389)

45

An expression vector was constructed in which DNA encoding the HSA secretion signal and mature HSA up to and including the 389th amino acid, lysine, was placed downstream of the *S.cerevisiae* phosphoglycerate kinase gene (PGK) promoter and followed by a stop codon and the PGK terminator of transcription. This vector was then introduced into *S.cerevisiae* by transformation and directed the expression and secretion from the cells of a molecule representing the N-terminal 389 amino acids of HSA.

50 An oligonucleotide was synthesised (Linker 1) which represented a part of the known HSA coding sequence (Figure 2) from the *Pst*I site (1092, Figure 2) to the codon for valine 381 wherein that codon was changed from GTG to GTC:

55

Linker 1

D P H E C Y A K V F D E

5' GAT CCT CAT GAA TGC TAT GCC AAA GTG TTC GAT GAA

3' ACGT CTA GGA GTA CTT ACG ATA CGG TTT CAC AAG CTA CTT

5 1100 1120

F K P L V

10 TTT AAA CCT CTT GTC 3'

AAA TTT GGA GAA CAG 5'

Linker 1 was ligated into the vector M13mp19 (Norlander *et al.* 1983) which had been digested with PstI 15 and HincII and the ligation mixture was used to transfet E.coli strain XL1-Blue (Stratagene Cloning Systems, San Diego, CA). Recombinant clones were identified by their failure to evolve a blue colour on medium containing the chromogenic indicator X-gal (5-bromo-4-chloro-3-indolyl- β -D-galactoside) in the presence of IPTG (isopropylthio- β -galactoside). DNA sequence analysis of template DNA prepared from bacteriophage particles of recombinant clones identified a molecule with the required DNA sequence, 20 designated mHOB12 (Figure 3).

M13mp19.7 consists of the coding region of mature HSA in M13mp19 (Norlander *et al.* 1983) such that the codon for the first amino acid of HSA, GAT, overlaps a unique Xhol site thus:

Asp Ala

25 5' C T C G A G A T G C A 3'

3' G A G C T C T A C G T 5'

Xhol

30 (EPA No. 210239 A1). M13mp19.7 was digested with Xhol, made flush-ended by S1-nuclease treatment and was then ligated with the following oligonucleotide (Linker 2):

35 Linker 2

40 5' T C T T T T A T C C A A G C T T G G A T A A A A G A 3'

3' A G A A A A T A G G T T C G A A C C T A T T T T C T 5'

HindIII

45 The ligation mix was then used to transfet E.coli XL1-Blue and template DNA was prepared from several plaques and then analysed by DNA sequencing to identify a clone, pDBD1 (Figure 4), with the correct sequence.

A 1.1 kb HindIII to PstI fragment representing the 5' end of the HSA coding region and one half of the 50 inserted oligonucleotide linker was isolated from pDBD1 by agarose gel electrophoresis. This fragment was then ligated with double stranded mHOB12 previously digested with HindIII and PstI and the ligation mix was then used to transfet E.coli XL1-Blue. Single stranded template DNA was prepared from mature bacteriophage particles of several plaques. The DNA was made double stranded *in vitro* by extension from annealed sequencing primer with the Klenow fragment of DNA polymerase I in the presence of deoxyribonucleoside triphosphates. Restriction enzyme analysis of this DNA permitted the identification of a clone 55 with the correct configuration, mHOB15 (Figure 4).

The following oligonucleotide (Linker 3) represents from the codon for the 382nd amino acids of mature HSA (glutamate, GAA) to the codon for lysine 389 which is followed by a stop codon (TAA) and a HindIII site and then a BamHI cohesive end:

Linker 3

5 E E P Q N L I K J
 5' GAA GAG CCT CAG AAT TTA ATC AAA TAA GCTTG 3'
 3' CTT CTC GGA GTC TTA AAT TAG TTT ATT CGAACCTAG 5'

10 This was ligated into double stranded mHOB15, previously digested with HincII and BamHI. After ligation, the DNA was digested with HincII to destroy all non-recombinant molecules and then used to transfect E.coli XL1-Blue. Single stranded DNA was prepared from bacteriophage particles of a number of clones and subjected to DNA sequence analysis. One clone having the correct DNA sequence was designated mHOB16 (Figure 4).

15 A molecule in which the mature HSA coding region was fused to the HSA secretion signal was created by insertion of Linker 4:

Linker 4

20

 M K W V S F I S L L F L
 5' GATCC ATG AAG TGG GTA AGC TTT ATT TCC CTT CTT TTT CTC
 25 G TAC TCC ACC CAT TCG AAA TAA AGG GAA GAA AAA GAG

 F S S A Y S R G V F R R
 30 TTT AGC TCG GCT TAT TCC AGG GGT GTG TTT CG 3'
 AAA ACG AGC CGA ATA AGG TCC CCA CAC AAA GCAGCT 5'

35 into BAMHI and Xhol digested M13mp19.7 to form pDBD2 (Figure 5). In this linker the codon for the fourth amino acid after the initial methionine, ACC for threonine in the HSA pre-pro leader sequence (Lawn *et al.*, 1981), has been changed to AGC for serine to create a HindIII site.

40 The 5' end of this construction was removed as a BamHI to PvuII fragment and ligated with the PvuII to BamHI fragment of double stranded mHOB16 (representing the 3' end of the truncated HSA gene) into pMA91 (Mellor *et al.*, 1983) at the BglII site to form pHOB31 (Figure 4). This molecule contains the truncated HSA coding region with the HSA secretion signal between the S.cerevisiae PGK gene promotor and terminator such that the 5' end of the gene abuts the promoter. The molecule also contains a selectable marker for yeast transformation, LEU2, and part of the yeast 2 μ m plasmid to permit autonomous replication in yeast.

45 The plasmid pHOB31 was introduced into S.cerevisiae AH22 (Hinnen *et al.*, 1978) by transformation using standard procedures (Beggs, 1978). Purified transformants were grown in YEPD broth (1% yeast extract, 2% peptone, 2% glucose) for 3 days at 30°C and the culture supernatant was then analysed, successfully, for the presence of HSA-related material by rocket gel electrophoresis. Figure 5 shows the electrophoretogram; the yield of HSA-related material from transformants harbouring a plasmid encoding HSA(1-389) is demonstrably higher than the yield from a transformant secreting mature, natural, HSA.

50 However, production of HSA (1-389) gave a product indistinguishable from HSA (1-387) (see Example 2) by both amino-terminal and carboxy-terminal sequence analysis. This is probably explained by the efficient removal of the COOH-terminal sequence Ile-Lys.

55 EXAMPLE 2: HSA (1-387)

The construction of a plasmid encoding HSA (1-387) was identical to the procedure for construction of the HSA (1-389) plasmid, pHOB31, except that the linker 3 was substituted by linker 5 (shown below) which represents the region from the codon for the 382nd amino acid of mature HSA (glutamate, GAA) to the

codon for leucine 387 which is followed by a stop codon and a HindIII site and then a BamHI cohesive end:

5 Linker 5

5	E E P Q N L Stop
	5' GAA GAG CCT CAG AAT TTA TAA GCTTG 3'
10	3' CTT CTC GGA GTC TTA AAT ATT CGAACCTAG 5'

The remainder of the construction was as detailed above for pHOB31 and resulted in the plasmid pDBD5.

15 EXAMPLE 3: (1-369)

In order to construct a plasmid encoding HSA (1-369), a linker was synthesised representing the region from the PstI site of mature HSA (position 1092, Figure 3) to the codon for cystine 369 which was followed by a stop codon (TAA), a HindIII site and then a BamHI cohesive end:

20

Linker 6

25	D P H E C Stop
	5' GAT CCT CAT GAA TGC TAA GCTTG
	3' A CGT CTA GGA GTA CTT ACG ATT CGAACCTAG

30 This linker was ligated with the BamHI PstI fragment of pDBD2, representing the 5' part of preproHSA, into pMA91 at the BglII site. A plasmid with the correct configuration was termed pDBD3 (Figure 6).

Production of HSA (1-369) by culturing S.cerevisiae transformed with pDBD3 gave low yields, indicating that the product may have been unstable in the yeast expression system used.

35

EXAMPLE 4: HSA (1-419)

40 For the construction of a plasmid encoding HSA (1-419) the BamHI - HincII fragment of pDBD2 was ligated with an annealed self-complementary oligonucleotide (linker 7):

Linker 7

45 5' ATAAGCTTGGATCCAAGCTTAT 3'

and then the ligation mix was digested with BamHI and the fragment was ligated into pMA91 to give pDBD4 (Figure 7). In this construct the HincII site (1256, Figure 3) of pDBD2 creates a blunt end after the second base of the codon for serine 419 and this codon is reformed by the linker 6 such that this codon is followed by a stop codon, a HindIII site and a BamHI site.

50 Expression of HSA (1-419) via plasmid pDBD5 in S.cerevisiae produced a molecule with the correct amino terminal sequence (Asp-Ala-His.....) but leucine and not serine was the COOH-terminal residue. Attempts to isolate the COOH-terminal peptide using a covalent label which should attach to cysteine 392 also were unsuccessful. It was concluded that proteolysis of part of the COOH-terminus of HSA (1-419) occurred. This is consistent with the observation of a small percentage of proteolysis in the same position of full-length HSA produced in an analogous manner in yeast (Sleep et al. 1988).

EXAMPLE 5: Fermentation of HSA(1-n)plus-producing yeast

A laboratory fermenter is filled to half its nominal working volume with an initial "batch" medium containing 50ml/l of a salts mixture (containing 114g/l KH_2PO_4 , 12g/l $\text{MgSO}_4 \cdot 3.0\text{g/l CaCl}_2 \cdot 6\text{H}_2\text{O}$, 2.0g/l Na_2EDTA : 10ml/l of a trace elements solution containing 3g/l $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 10g/l $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 3.2g/l $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 79mg/l $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 1.5g/l H_3BO_3 , 0.2g/l KI , 0.5g/l $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 0.56g/l $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 5 75ml/l H_3PO_4 : 20g/l sucrose: 50ml/l of a vitamins mixture containing 1.6g/l Ca pantothenate, 1.2g/l nicotinic acid, 12.8g/l inositol, 0.32g/l thiamine HCl and 8mg/l pyridoxine HCl and 8mg/l biotin. An equal volume of "feed" medium containing 100ml/l of the salts mixture, 20ml/l of trace elements solution 500g/l sucrose and 100ml/l vitamin solution is held in a separate reservoir connected to the fermenter by a metering pump.

10 The fermenter is inoculated with *Saccharomyces cerevisiae* which has been transformed as above with plasmid pDBD3 from Example 2. The pH is maintained at 5.7 ± 0.2 by automatic addition of ammonia or sulphuric acid, the temperature is kept at 30 °C and the stirred speed is adjusted to give a dissolved oxygen tension (DOT) of > 20% air saturation at 1 v/v/min air flow rate. When the initial substrate has been consumed, the metering pump is turned on, maintaining a growth rate of approximately 0.15h^{-1} . The pump rate is increased to maintain this growth rate until the stirrer speed reached its maximum value at which point it is not possible to increase the pump rate any further without causing the DOT to fall below 15% air saturation which is the minimum value permitted to occur. PPG 2000 is added in response to a foam sensor. None is added until over 50% of the feed solution had been added. The final level of addition is 0.2g/l.

15 20 HSA(1-387) is secreted into the medium

EXAMPLE 6: Binding of bilirubin to HSA(1-387)

25 Binding of the haem metabolite, bilirubin, to HSA (1-387) was carried out by a fluorescence enhancement method (Beaven and Gratzen (1973) *Eur. J. Biochem.* **33**, 500-510). Figure 8 shows that the enhancement of bilirubin fluorescence as a function of protein/bilirubin ratio is indistinguishable for HSA(1-387) and clinical grade HSA.

30 The interaction of HSA and bilirubin is very sensitive to the conformation of the protein (Beaven and Gratzen, loc. cit.) and these results indicate that no gross alteration in conformation of the regions of HSA represented by HSA(1-387) has occurred through the expression of a shorter molecule.

EXAMPLE 7: Oncotic behaviour of HSA(1-387)

35 HSA(1-387) was concentrated in 0.9% w/v saline to a final protein concentration of 54 mg/ml. Dilutions of this concentrate, together with dilutions of a clinical grade HSA (100 mg/ml), were compared for osmotic effect in a colloid osmometer. Figure 9 indicates that HSA(1-387) gives a colloid osmotic pressure approximately one-third higher than that of full-length HSA at a given protein concentration. Importantly, the 40 increase in colloid osmotic pressure with protein concentration is approximately linear over a range up to 5% w/v, which represents the concentration in plasma.

This indicates that HSA(1-387) does not self-associate appreciably within a useful working clinical concentration range.

45 EXAMPLE 8: Formulations for Injection

The HSA(1-n)plus of the invention may be presented in container sizes ranging from 20ml to 500ml, with the concentration thereof varying (typically) from 2% to 17%, for example 3%, 13% or 17%.

50 The solution for administration is sterile and pyrogen free. A 3% solution is osmotically similar to human plasma. At least 96% of the total protein is preferably albumin. The sodium ion content is generally between 130-160mmol/litre and the potassium ion content is generally not more than 2mmol/litre. The pH is adjusted to 6.9 ± 0.5 . The concentration of citrate is generally no more than 20mmol/litre and may be absent altogether.

55 Stabilizers may be used, for example either 0.16 millimole sodium acetyl tryptophanate, or 0.08 millimole sodium acetyl tryptophanate and 0.08 millimole sodium caprylate per gram of HSA(1-n)plus.

References

5 Beggs, J.D. (1978). Nature. 275, 104-109.
 Brown, J.R. and Shockley, P., (1982) in "Lipid-Protein Interactions" 1, 25-68. Eds. Hayes, O. and Jost, P.C.
 6 Hinnen, A. et al (1978). Proc. Natl. Acad. Sci. USA, 75, 1929-1933.
 Lawn, R.M. et al (1981). Nucl. Acid. Res. 9, 6103-6114.
 Maniatis, T. et al (1982). Molecular cloning: A laboratory manual. Cold Spring Harbour Laboratory, Cold
 Spring Harbor, New York.
 Mellor, J. et al (1983). Gene, 24, 1-14.
 10 Messing, J. (1983). Methods Enzymol. 101, 20-78.
 Norrander, J. et al (1983). Gene, 26, 101-106.
 Sanger, F. et al (1977). Proc. Natl. Acad. Sci. USA, 74, 5463-5467.
 Sleep, D. Bellfield, G.P. and Goodey, A.R. (1988) Yeast 4, S168.

15

Claims

1. A polypeptide comprising the N-terminal portion of mature human serum albumin up to amino acid residue n, where n is 369 to 419, but not 387, and variants thereof.
 20 2. A polypeptide according to Claim 1 wherein the polypeptide is selected from the group consisting of HSA (1-373), HSA (1-388), HSA (1-389), HSA (1-390) and HSA (1-407) and variants thereof.
 3. A pharmaceutical composition comprising a polypeptide according to Claim 1 or 2 except that n may be 387.
 4. A composition according to Claim 3 wherein the polypeptide is HSA (1-387) or a variant thereof.
 25 5. A nucleotide sequence encoding a polypeptide comprising the N-terminal portion of mature human serum albumin up to amino acid residue n, where n is 309 to 419, and polypeptide variants thereof, the nucleotide sequence not being linked at its 3' end to a further sequence encoding the C-terminal portion of mature human serum albumin from amino acid residue n + 1 to 585.
 6. A nucleotide sequence according to Claim 5 wherein n is 387.
 30 7. A nucleotide sequence according to Claim 5 or 6 linked at its 5' end to a further nucleotide sequence encoding a peptide corresponding to the pro-, pre-, or pre-pro- position of HSA, a methionine residue, or another leader sequence.
 8. An expression vector suitable for transformation of and expression in a selected host, the vector comprising a nucleotide sequence according to any one of Claims 6 and 8 and the said nucleotide sequence being a DNA sequence.
 35 9. A host organism transformed with a vector according to Claim 8.
 10. A host organism according to Claim 9 which is Saccharomyces cerevisiae.
 11. A process for the production of a polypeptide comprising the culture under suitable conditions of a host microorganism according to Claim 9 or 10, the said polypeptide being encoded by the said nucleotide sequence.
 40 12. A laboratory medium for the growth of microorganisms comprising a polypeptide according to Claim 1, except that n may be 387.
 13. A medium according to Claim 12 wherein n is 387.

45

50

55

FIGURE 1

10 20

Asp Ala His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys

30 40

Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val

50 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu

70 80

Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu

90 100

Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu

110 120

Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val

130 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr

150 160

Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg

170 180

Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro

190 200

Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys

210 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser

230 240

Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys

250 260

Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu

270 280

Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu

290 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala

310 320

Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala

330 340

Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp

350 360

Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys

370 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu

FIGURE 1 Cont.

390	400
Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu	
410	420
Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr	
430	440
Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His	
450	460
Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu	
470	480
Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser	
490	500
Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys	
510	520
Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu	
530	540
Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr	
550	560
Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys	
570	580
Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln	
Ala Ala Leu Gly Leu	

FIGURE 2 DNA sequence coding for mature HSA

10	20	30	40	50	60	70	80
GATGCACACAAGAGTGAGGTGCTCATCGGTTAAAGATTGGGAGAAGAAAATTCAAAGCCTGGTGTGATTGCCTT							
D	A	H	K	S	E	V	A
H	R	F	K	D	L	G	E
K	F	K	D	L	G	E	N
A	L	V	L	I	A	F	
90	100	110	120	130	140	150	160
TGCTCACTATCTCAGCAGTGTCCATTGAAAGATCATGTAAGATGAAGTAACGTGAATTTGCAAAAACATGTG							
A	Q	Y	L	Q	Q	C	P
Y	L	Q	Q	C	P	F	E
Z	D	H	V	K	L	V	N
E	V	T	E	F	A	K	T
170	180	190	200	210	220	230	240
TTGCTGATGAGTCAGCTGAAATTGTGACAAATCACCTACCCCTTTGGAGACAAATTATGCACAGTTGCAACTCTT							
V	A	D	E	S	A	E	N
D	S	A	E	N	C	D	K
S	L	H	T	L	F	G	D
L	H	T	L	F	K	L	C
H	T	L	F	K	C	T	V
T	L	F	K	C	T	V	A
250	260	270	280	290	300	310	320
CGTGAACCTATGGTGAATGGCTGACTGCTGTGCAAAACAAGAACCTGAGAGAAATGAATGCTCTTGCAACACAAAGA							
R	E	T	Y	G	E	M	A
E	T	Y	G	E	M	A	D
T	Y	G	E	M	A	D	C
Y	G	E	M	A	D	C	C
G	E	M	A	D	C	A	K
E	M	A	D	C	A	K	Q
M	A	D	C	A	K	Q	E
A	D	C	A	K	Q	E	P
D	C	A	K	Q	E	P	E
A	K	Q	E	P	E	R	N
K	Q	E	P	E	R	N	E
Q	E	P	E	R	N	E	C
E	P	E	R	N	E	C	F
P	E	R	N	E	C	F	L
E	R	N	E	C	F	L	Q
R	N	E	C	F	L	Q	H
N	E	C	F	L	Q	H	K
E	C	F	L	Q	H	K	D
330	340	350	360	370	380	390	400
TGACAACCCAAACCTCCCCCGATTGGTGAGACCAGAGGTTGATGTGACTGCTTTCATGACAATGAAGAGACAT							
D	N	P	N	L	P	R	L
N	P	N	L	P	R	L	V
P	N	L	P	R	L	V	R
N	L	P	R	L	V	R	P
L	P	R	L	V	R	P	E
P	R	L	V	R	P	E	V
R	L	V	R	P	E	V	D
L	V	R	P	E	V	D	V
V	R	P	E	V	D	V	M
R	P	E	V	D	V	M	C
P	E	V	D	V	M	C	T
E	V	D	V	M	C	T	A
V	D	V	M	C	T	A	F
D	V	M	C	T	A	F	H
V	M	C	T	A	F	H	D
M	C	T	A	F	H	D	N
C	T	A	F	H	D	N	E
T	A	F	H	D	N	E	E
A	F	H	D	N	E	E	T
410	420	430	440	450	460	470	480
TTTGAAAAAAACTTATGAAATTGCCAGAAGACATCCTTACTTTATGCCCGGAACTCCTTTCTTGCTAAAAGG							
F	L	K	K	Y	L	Y	E
L	K	K	Y	L	Y	E	I
K	K	Y	L	Y	E	I	A
K	Y	L	Y	E	I	A	R
Y	L	Y	E	I	A	R	R
L	Y	E	I	A	R	R	H
Y	E	I	A	R	R	H	P
E	I	A	R	R	H	P	Y
I	A	R	R	H	P	Y	F
A	R	R	H	P	Y	F	Y
R	R	H	P	Y	F	Y	A
R	H	P	Y	F	Y	A	P
H	P	Y	F	Y	A	P	E
P	Y	F	Y	A	P	E	L
Y	A	P	E	L	L	F	F
A	P	E	L	L	F	F	A
P	E	L	L	F	F	A	K
E	L	L	F	F	A	K	R
L	L	F	F	A	K	R	D
L	F	F	A	K	R	D	E
F	A	K	R	D	E	L	R
A	K	R	D	E	L	R	D
K	D	E	L	R	D	E	L
D	E	L	R	D	E	L	R
E	L	R	D	E	L	R	D
L	R	D	E	L	R	D	E
R	D	E	L	R	D	E	L
D	E	L	R	D	E	L	R
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D	E	L	R	D	E	L	R
E	L	R	D	E	L	R	D
L	R	D	E	L	R	D	E
R	D	E	L	R	D	E	L
D	E						

FIGURE 2 Cont.

1050 1060 1070 1080 1090 1100 1110 1120
 GAGACTGCCAAGACATATGAAACCACTCTAGAGAAGTGCCTGCCGCTGCAGATCCTCATGAATGCTATGCCAAAGTGT
 R L A K T Y E T T L E K C C A A A D P H E C Y A K V

 1130 1140 1150 1160 1170 1180 1190 1200
 TCGATGAATTAAACCTCTTGGAAGAGCCTCAGAATTAAATCAAACAAACTGTGAGCTTTTGAGCAGCTGGAGAG
 F D E F K P L V E E P Q N L I K Q N C E L F E Q L G E

 1210 1220 1230 1240 1250 1260 1270 1280
 TACAAATTCCAGAATGCGCTATTAGTCGTTACACCAAGAAAAGTACCCCAAGTGTCAACTCCAACCTTGTAGAGGTCTC
 Y K F Q N A L L V R Y T K K V P Q V S T P T L V E V S

 1290 1300 1310 1320 1330 1340 1350 1360
 AAGAAAACCTAGGAAAAGTGGGCAGCAAATGTTGAAACATCCTGAAGCAAAAGAATGCCCTGTGCAGAAGACTATCTAT
 R N L G K V G S K C C K H P E A K R M P C A E D Y L

 1370 1380 1390 1400 1410 1420 1430 1440
 CCGTGGTCCTGAACCCAGTTATGTTGCATGAGAAAACGCCAGTAAGTGACAGAGTCACAAAATGCTGCACAGAGTC
 S V V L N Q L C V L H E K T P V S D R V T K C C T E S

 1450 1460 1470 1480 1490 1500 1510 1520
 TTGGTGAACAGGGCACCAGCTTTCTGGAAAGTCGATGAAACATACGTTCCAAAGAGTTAATGCTGAAACATT
 L V N R R P C F S A L E V D E T Y V P K E F N A E T F

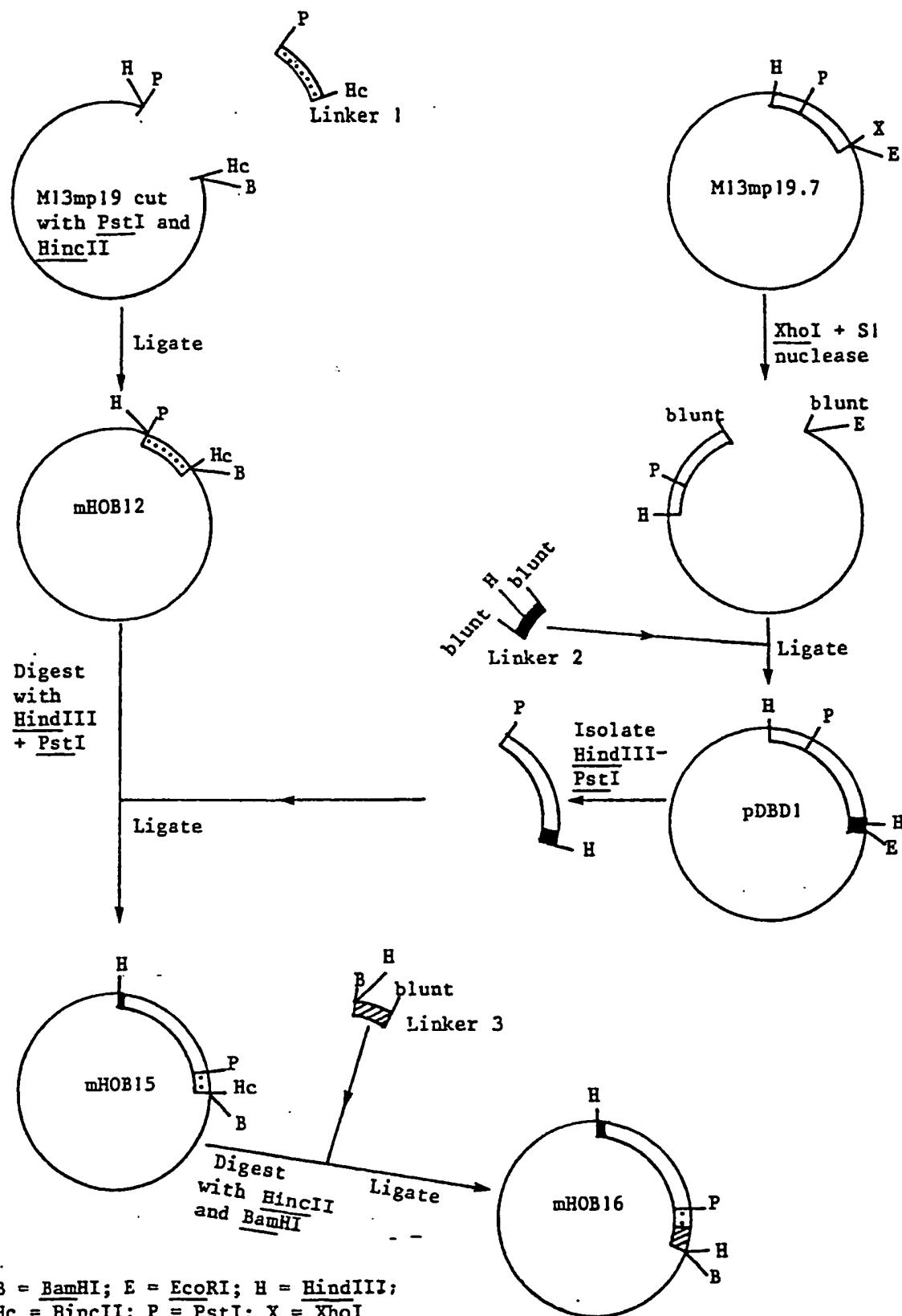
 1530 1540 1550 1560 1570 1580 1590 1600
 CACCTTCCATGCAGATATATGCACACTTCTGAGAAGGGAGAGACAAATCAAGAAACAAACTGCACTTGTGAGCTTGTGA
 T F H A D I C T L S E K E R Q I K K Q T A L V E L V

 1610 1620 1630 1640 1650 1660 1670 1680
 AACACAAGCCAAGGCAACAAAGAGCAACTGAAAGCTGTTATGGATGATTGCGAGCTTGTAGAGAAAGTGTGCAAG
 K H K P K A T K E Q L K A V M D D F A A F V E K C C K

 1690 1700 1710 1720 1730 1740 1750 1760
 GCTGACGATAAGGAGACCTGCTTGCCGAGGGTAAAAAAACTTGTGCTGCAAGTCAAGCTGCCCTAGGCTTATAACA
 A D D K E T C F A E E G K K L V A A S Q A A L G L

 1770 1780
 TCTACATTAAAAGCATCTCAG

FIGURE 3 Construction of mHOB16



B = BamHI; E = EcoRI; H = HindIII;
 Hc = BincII; P = PstI; X = XbaI

FIGURE 4 Construction of pHOB31

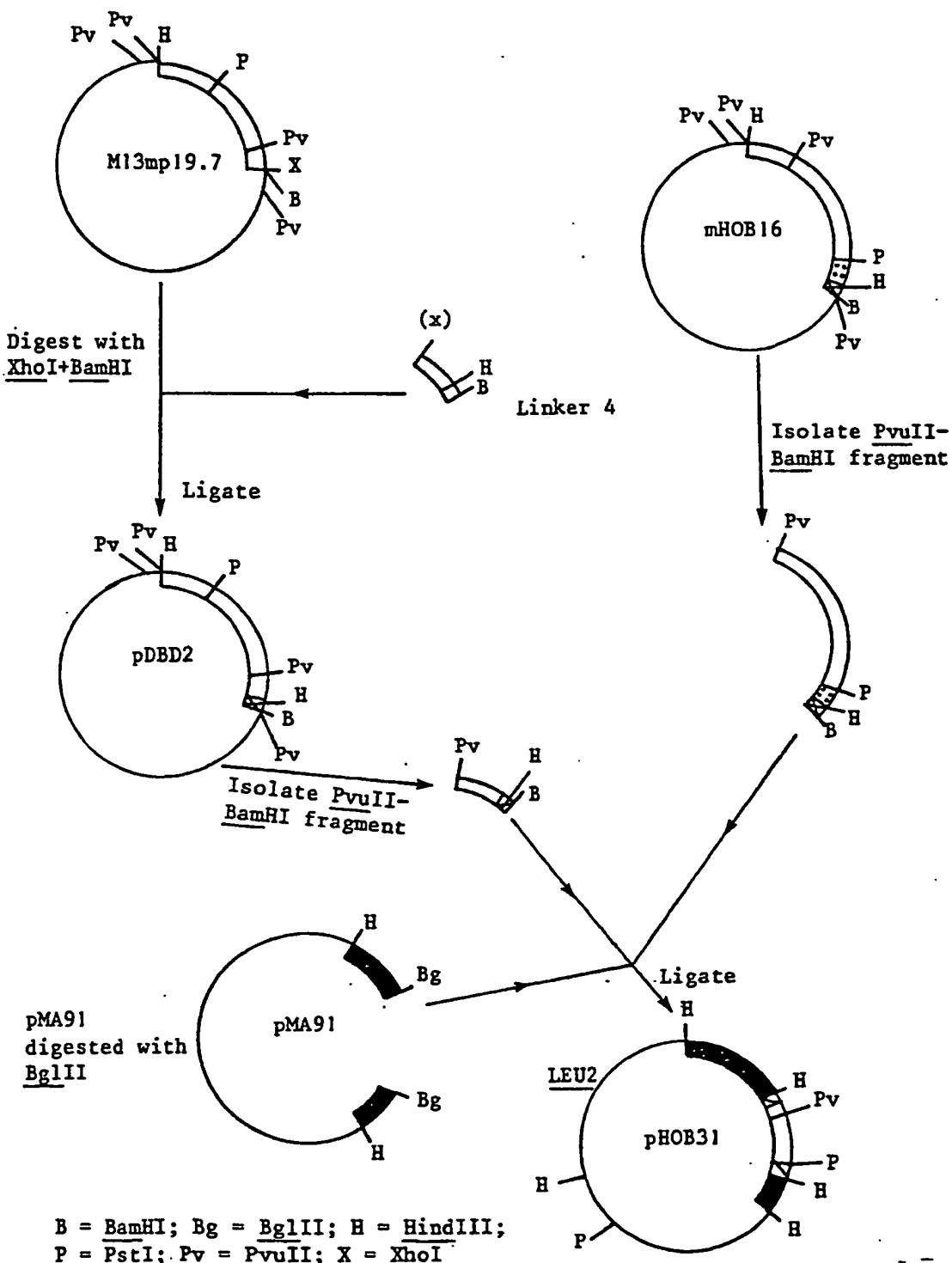
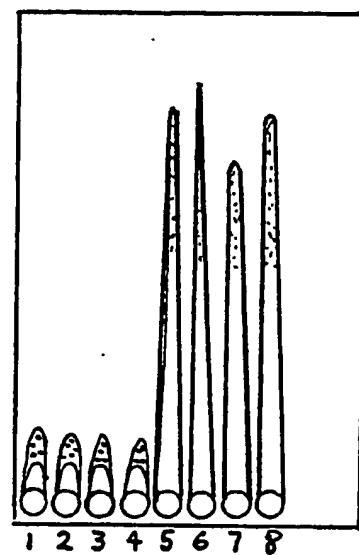


FIGURE 5

Rocket immunoelectrophoretic analysis of culture supernatant from *S.cerevisiae* AH22 transformants obtained with a plasmid containing the complete HSA coding region (samples 1-4) and from transformants harbouring an equivalent plasmid encoding truncated HSA (1-389) (samples 5-8).

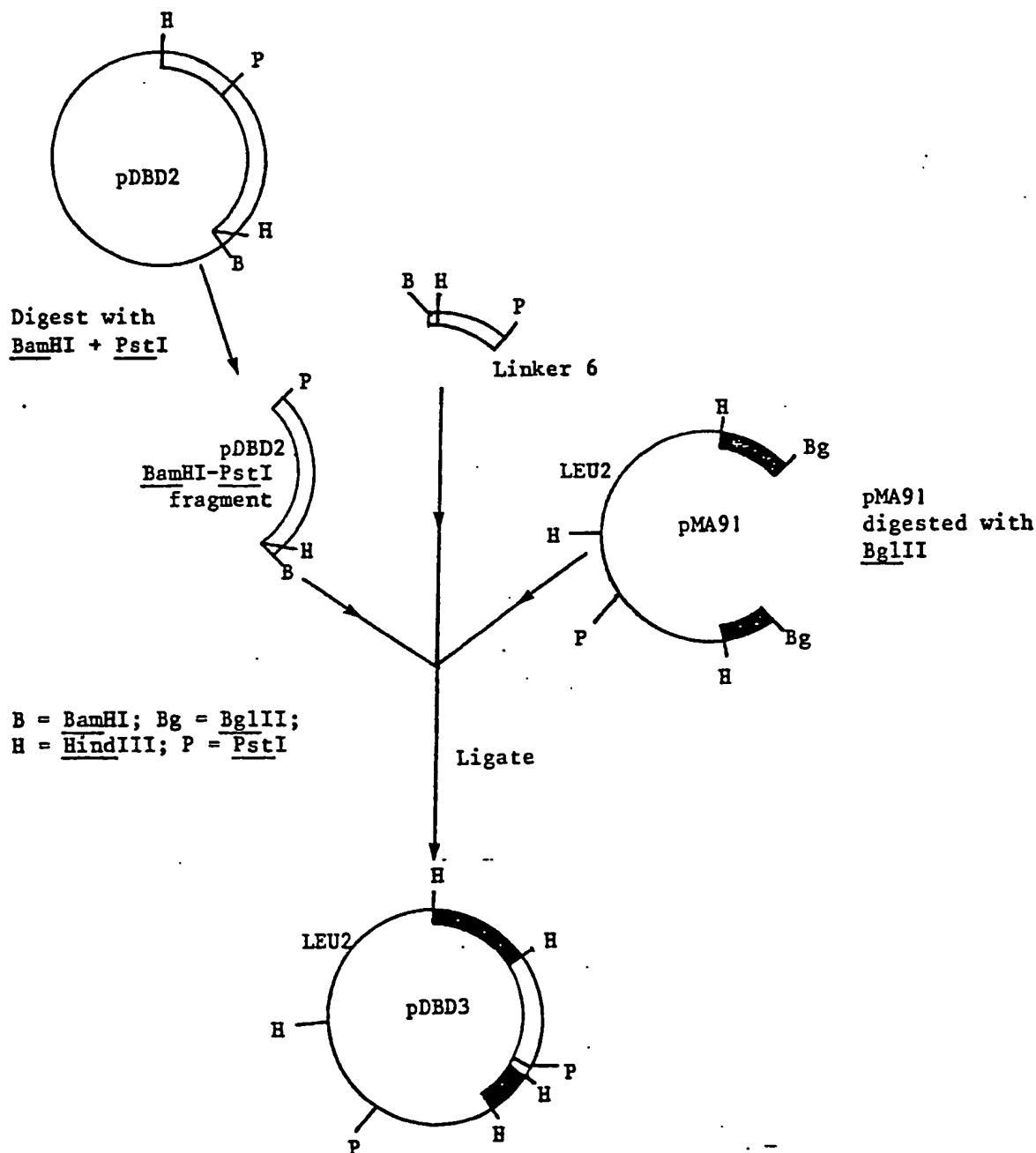
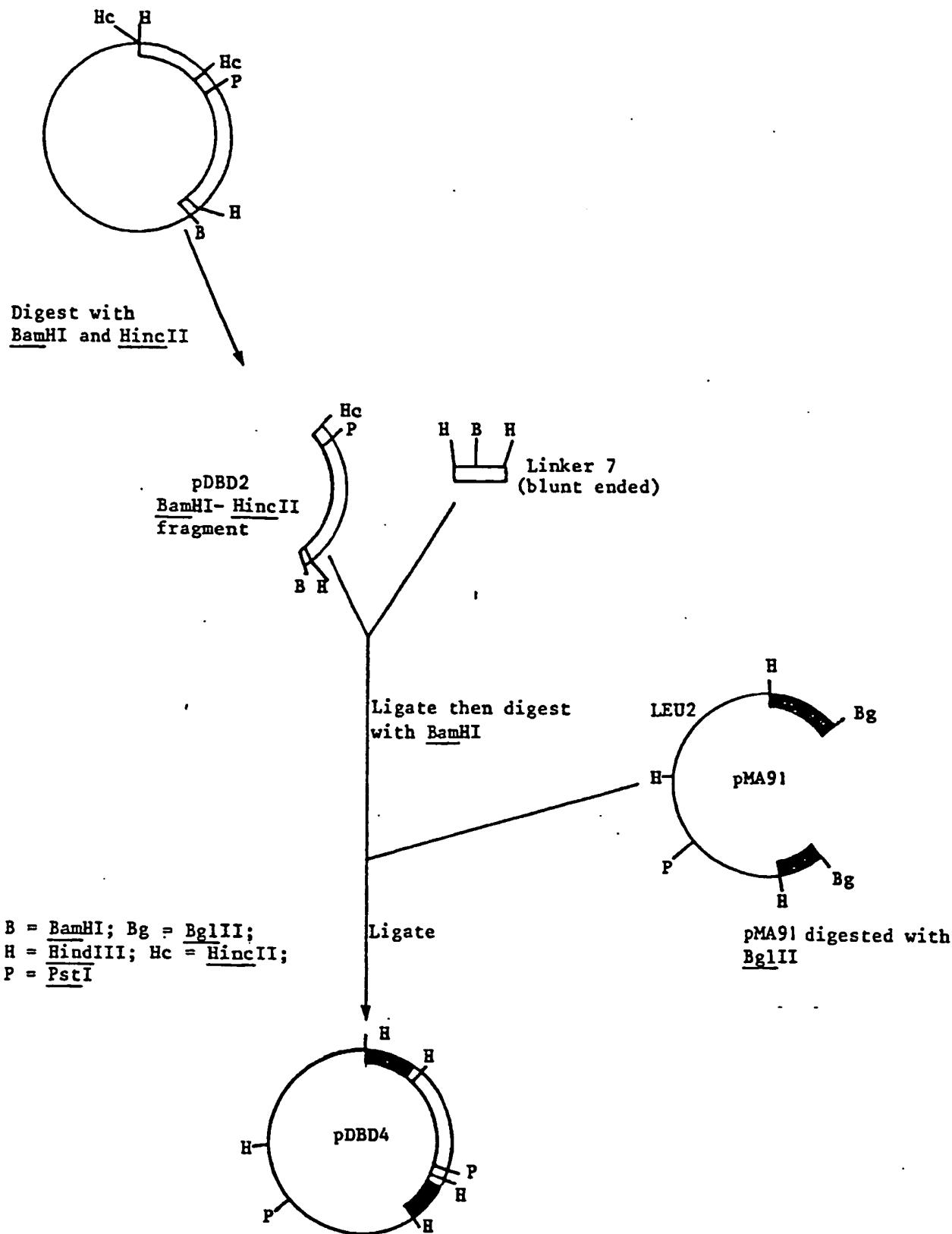
FIGURE 6 Construction of pDBD3

FIGURE 7 Construction of pDBD4

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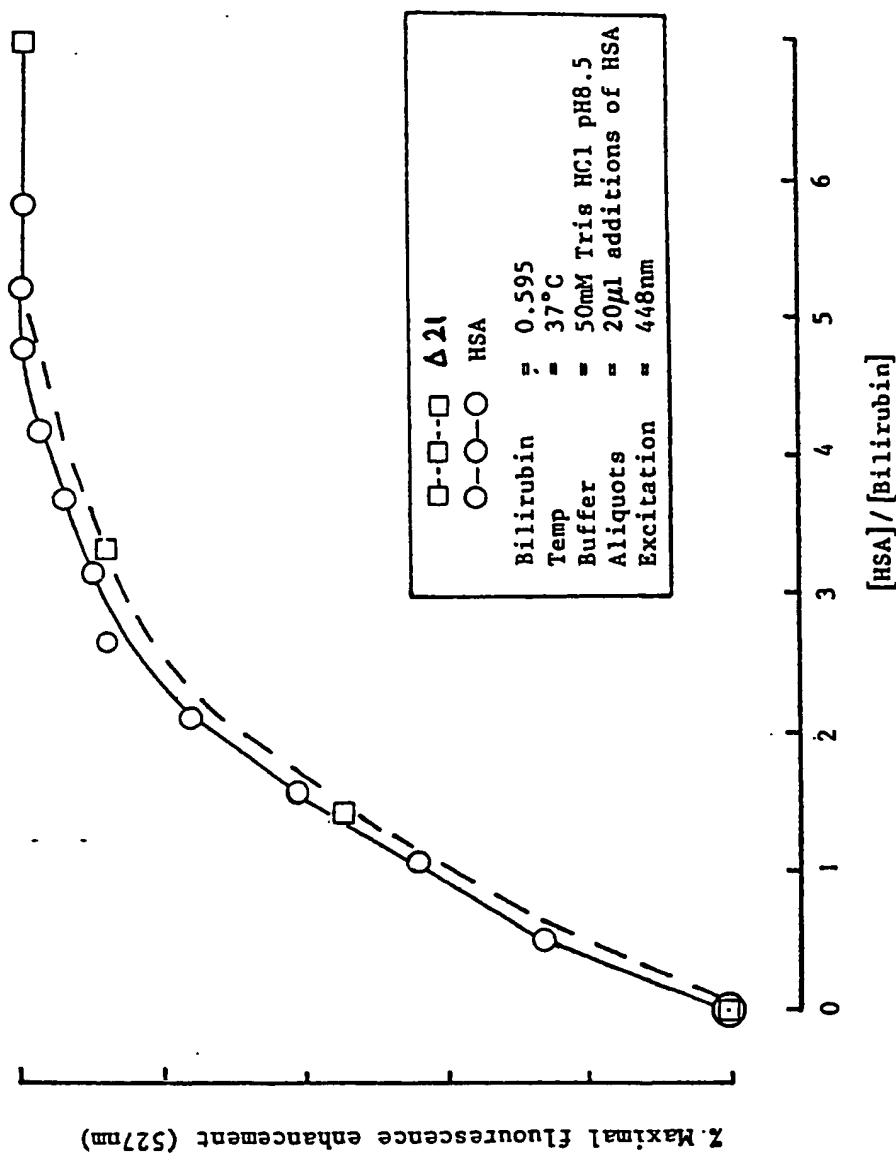
Neu eingerichtet: Newry neu
· à la veille pour déroulé



Neu eingereicht / Newly filed
Nouvellement déposé

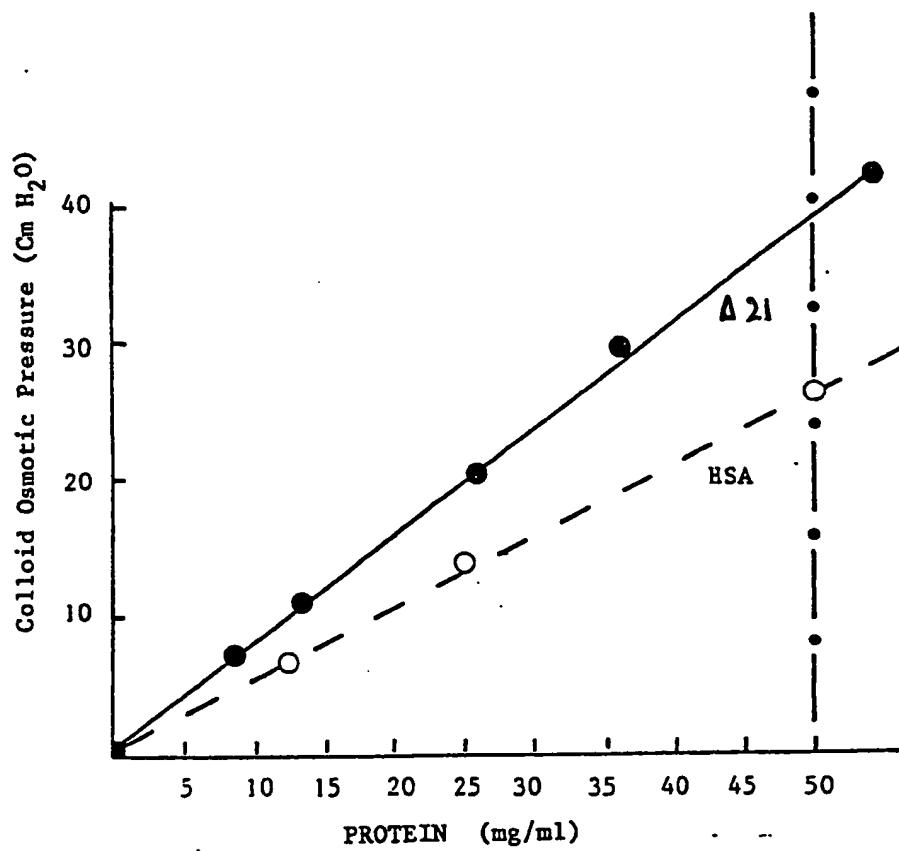
FIGURE 8

Titration of bilirubin by BPL HSA and $\Delta 21$
(measurement of fluorescence enhancement)



Neu eingereicht / Newly filed
Nouvellement déposé

FIGURE 9





EP 88 31 0000

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 4)
X,D	THE BIOCHEMICAL JOURNAL, vol. 163, no. 3, 1977, pages 477-484, GB; M.J. GEISOW et al.: "Physical and binding properties of large fragments of human serum albumin" * Abstract * ---	4	A 61 K 37/02 C 12 N 15/00 C 12 N 1/18 C 12 P 21/02 // C 12 N 5/00
A	THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 257, no. 6, 25th March 1982, pages 2770-2774, US; N. DOYEN et al.: "Immunochemical cross-reactivity between cyanogen bromide fragments of human serum albumin" * Page 2770, column 2, paragraph 1 * ---	1-9	
A	EP-A-0 073 646 (GENENTECH INC.) * Figure 2; page 12, lines 16-18 * ---	1-9,11	
A	EP-A-0 201 239 (DELTA BIOTECHNOLOGY LTD) * Figure 3; example II * -----	1-11	
			TECHNICAL FIELDS SEARCHED (Int. Cl. 4)
			C 12 N C 12 P
<p>The present search report has been drawn up for all claims</p>			
Place of search	Date of completion of the search		Examiner
THE HAGUE	09-02-1989		CUPIDO M.
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<p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p>			

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